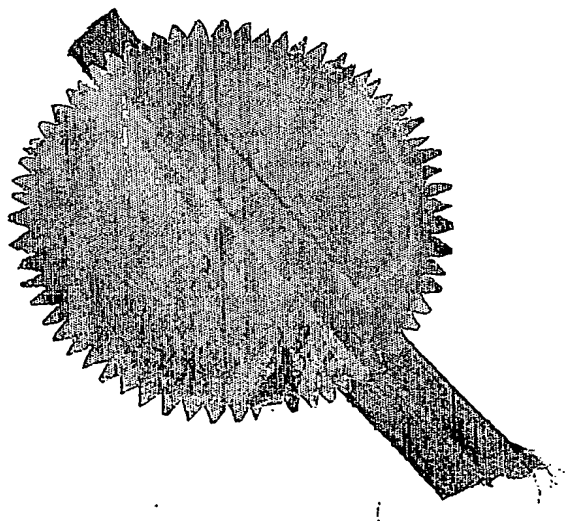
INTELLECTUAL PROPERTY **INDIA**

सत्यमेव जयते

GOVERNMENT OF INDIA
MINISTRY OF COMMERCE & INDUSTRY
PATENT OFFICE, DELHI BRANCH
W - 5, WEST PATEL NAGAR
NEW DELHI - 110 008.

I, the undersigned being an officer duly authorized in accordance with the provision of the Patent Act, 1970 hereby certify that annexed hereto is the true copy of the ***Application, Complete Specification*** *and* ***Drawing Sheets*** *filed in connection with Application for Patent No.****5/Del/2004*** ***dated 1st January 2004.***



Witness my hand this 8th day of July 2005.

(S.K. PANGASA)
Assistant Controller of Patents & Designs

0005 DEL 04

FORM 1

THE PATENT ACT, 1970
(39 of 1970)
APPLICATION FOR GRANT OF A PATENT

02 JAN 2004

BY THE ASSIGNEE AND LEGAL REPRESENTATIVE OF THE TRUE AND FIRST INVENTORS.

(See Section 7 , 5 (2), 54 and 135 , rule39)

We, PANACEA BIOTEC LIMITED of B-1 ,Extn. A/27 Mohan Co-operative, Indl. Estate, Mathura Road, New Delhi – 110044, A Company registered under " The Companies Act 1956.

hereby declare :-

(i) That we are in possession of an invention for "PHARMACEUTICAL COMPOSITIONS COMPRISING OF AN EXTRACT OF THE PLANT EUPHORBIA PROSTRATA FOR THE CONTROL AND TREATMENT OF ANORECTAL AND COLONIC DISEASES"

(ii) That the provisional specification relating to this invention is filed with this application

(iii) That there is no lawful ground of objection to the grant of a patent to us.

(iv) Further declare that the inventors for the said invention are

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(V) That we are assignee of the true and first inventors.

(v) That our address for service in India is as follows :

Nagpaul & Associates
Patent & Trade Mark Attorneys
5/10, West Patel Nagar
New Delhi – 110008.

(vi) Following declaration was given by the inventors :

We, the true and first inventors for this invention declare that the applicants herein are our assignee

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(vii) That the best of my knowledge, information and belief; the facts and matters stated herein are correct and there is no lawful ground of objection to the grant of patents.

(viii) Following are the attachments with the application.

a. Provisional Specification (3 copies)

b. Power of Authority.

c. Fee Rs. 3000/- by cheque bearing No. 905140 dated 27.12.2004 Drawn on UTIBANK LTD.

We request that a patent may be granted to us for the said invention.

Dated this 1st day of January, 2004.

For Panacea Biotec Limited.,

A.N. Nagpaul
Agent for the Applicants

TO,
THE CONTROLLER OF PATENTS
THE PATENT OFFICE

FORM 2 0005 DEL 04

12 JAN 2004

THE PATENTS ACT, 1970

COMPLETE SPECIFICATION

(SECTION 10)

Pharmaceutical compositions comprising of an extract of the plant *Euphorbia prostrata* for the control and treatment of anorectal and colonic diseases

FIELD OF THE INVENTION

The invention relates to a novel compositions comprising of an extract of the plant *Euphorbia prostrata* useful for the treatment of anorectal disease including hemorrhoids and colonic diseases. The novel compositions possess properties to control inflammation, prevent capillary bleeding and fragility in mammalians, particularly human beings.

BACKGROUND OF THE INVENTION

Among the various anorectal and colonic diseases, hemorrhoids occupy a prominent position and have been the subject of numerous clinical studies. Hemorrhoidal disease is characterised by bleeding, without any pain. Fresh blood spots occur immediately. on defecation. However, pain occurs when the hemorrhoids are secondarily infected, or complicated by thrombosis and anal fissures. Hemorrhoids can be caused by a variety of factors including hormones, genes, inflammation. infection. constipation, exercise, vascular stasis, diet. strain, physical stance in defecation. loss of connective tissue elasticity with age etc. The symptoms most widely recognized are bleeding. pain and prolapse (Hyams and Philpot. 1970; Smith. 1987). These may be accompanied by thrombosis. pruritis, edema etc. Hemorrhoids can be treated through reduction of inflammation and pain, haemostasis, wound healing and protection of vascular walls. Thus, an effective treatment of acute hemorrhoidal attacks should not only provide relief as early as 2-3 days after initiation of the treatment, but also reduce the recurrence of such attacks.

There exist several procedures for the treatment of hemorrhoids. WO8803398 patent application discloses surgical dressings for such treatment. Patents have been granted in respect of surgical devices such as European patent no. 0095142. U.S. Patent no. 4,621,635 has been granted for the use of lasers in the treatment of hemorrhoids. The techniques of cryopharmacotherapy and electrochemical techniques for treatment of hemorrhoids have also been patented vide European patent no. 0091405 and European patent no. 0116688, respectively. However, the biggest drawbacks of the above are the involvement of medical experts beyond mere prescription of medicines and probable hospitalization. Also, some of them are physically and/or psychologically unpleasant in application.

Several patents (U.S. patent nos. 4,160,148, 4,508,728, 4,797,392, 4,518,583 and 5,234,914) have been granted in respect of compositions containing certain wound healing

agents to provide symptomatic relief, by promoting tissue repair, reducing inflammation and encouraging wound healing. Some of them like U.S. patent nos. 4,518,583 and 5,234,914 contain antimicrobial agents. These compositions, however, only relieve symptoms associated with inflammation, like heat, itching, redness, pain and swelling.

A number of compositions for the treatment of anorectal diseases (including hemorrhoids) are based on the anesthetic and vasoconstrictive properties of the constituents, but these provide only temporary symptomatic relief.

Patents in the United States of America (U.S. patent nos. 4,613,498, 4,626,433, 5,166,132, 5,219,880, 5,234,914 and 4,797,392) and Europe (European patents nos. 0225832 and 0513442) have been granted in respect of compositions with varying constituents, for topical application in the form of suitable and acceptable pharmaceutical carriers, such as salts, ointments, etc., with organic, inorganic or biological active agents. However, these compositions provide only temporary relief and are limited to local application and cannot be used for systemic use or oral administration.

A topical treatment for hemorrhoidal pain and for spasms of the sphincters and muscles located in the GI tract is disclosed in a granted patent (U.S. patent no. 5,595,753), which includes amino acid L-arginine in a pharmaceutically acceptable carrier. Another U.S. patent no. 5,591,436 has been granted for a composition for dietary supplement for the treatment of hemorrhoids. The composition comprises 60% to 95% Indian Barberry by weight; 4.8% to 38% Nagkesar by weight; and 0.2% to 2% Margosa tree leaves by weight.

Another U.S. patent no. 5,562,906 discloses the use of bark or berries of the species *Xanthoxylum clava herculis* L and *Xanthoxylum americanum* Hill, both of the yellow wood tree family, are employed for the treatment of hemorrhoids and other membrane and capillary disorders of the veins and arteries. Improved strength and flexibility of the veins, arteries and their constituent structures is obtained.

The flavonoidal constituents present in the extract of *Euphorbia prostrata* are reported to have anti-inflammatory properties. The phenolic compounds like ellagic and gallic acids and tannins are reported to have anti-inflammatory, haemostatic, gastro-protective and wound healing properties.

Other plants containing flavonoids including apigenin glycosides and luteolin glycosides are *Ixora arborea* (Rubiaceae), *Bommervia hispida* (Pteridaceae), *Adenocalymma alliaceum*

(Bignomiaceae), *Thalictrum thunbergii* (Renunculaceae), *Perilla frutescens* (Labiateae), *Chrysenthemum indicum, C. coronarium* and *Matricaria chamomilla* (Compositae). *Thymus membranaceous* (Labiateae). *Digitalis lanata* (Scrophulariaceae), *Cuminum cyminum* and *Petroselinum* (Umbelliferae). Several species of *Euphorbia* like *Euphorbia minuta, Euphorbia microfolia, Euphorbia granulata* (Euphorbiaceae) contain both apigenin and luteolin. Ellagic acid and other phenolic acids have been reported from different species of *Euphorbia.*

The safety of various components of the *Euphorbia* extract has been reported in the literature. Some of the reports also claim anti-mutagenic/ anti-carcinogenic/ anti-genotoxic properties of the components of the *Euphorbia* extract.

An Indian patent no. 186803 and several other patents (Australia, No. 698407; China, No. CN 1102387C; Europe, No. 868914; Russia, No. 2174396; South Africa, No. 97/2900; South Korea, No. 281679 and U.S., No. 5,858,371) have been granted to this applicant for a composition comprising a flavonoid containing extract of *Euphorbia prostrata* for treatment of anorectal and colonic diseases. The claimed extract comprises of very high contents of flavonoids (35 – 62% by weight), which is very expensive and time consuming to purify. Further, the presence of the phenolic compounds that are therapeutically useful for treatment of anorectal and colonic diseases due to their hemostatic and astringent properties, has not been established in the claimed extract. The presence of the phenolic compounds like ellagic acid, gallic acid and tannins comprising of these acids makes the claimed extract more effective for treatment of hemorrhoids and other colonic diseases, as the phenolic compounds are known gastroprotective agents. The antimicrobial properties of these phenolic compounds further prevent secondary infections often accompanied with hemorrhoids, fissures, fistulas etc.

Furthermore, the process of extraction described in the said patent application comprises a step of treatment of the combined and concentrated extract with hot water and then taking the water-soluble portion for obtaining the flavonoidal components. It was discovered in the present invention that the water- soluble portion contains substantial amount of phenolic compounds. The new method of the present invention comprises washing of the extract with hexane where only waxy materials and pigments are removed and there is no significant loss of phenolic compounds.

The inventors have further researched, and have found that the novel flavonoid and phenolic compounds containing extract of *Euphorbia prostrata* disclosed in the present invention

exhibits improved pharmacological response in comparison to existing compositions employing flavonoids either from *Euphorbia* or other sources. Further, the extraction procedure of the disclosed *Euphorbia prostrata* extract in the present invention is more cost effective and less time consuming in comparison to that of existing compositions employing flavonoids isolated from *Euphorbia prostrata*. The commercial implications of the improved and economic extraction procedure led the inventors to re-establish the pharmacological and toxicological validity of the new extract. The results were strikingly better than those of the equivalent flavonoid doses of the more purified extract of *Euphorbia prostrata* as disclosed earlier.

The present invention provides a pharmaceutical composition that is safe and painless and has long-term effectiveness.

OBJECTIVE OF THE INVENTION

An objective of the present invention is to provide compositions for treating anorectal diseases including hemorrhoids and colonic diseases.

Another objective for the present invention is to provide compositions for treating anorectal and colonic diseases including hemorrhoids comprising of an extract of the plant Euphorbia prostrata containing flavonoids and phenolic compounds, optionally with other therapeutic agent(s) and/or pharmaceutically acceptable carrier(s)/base(s).

SUMMARY OF THE INVENTION

A composition and a method for treating anorectal diseases including hemorrhoids and colonic diseases using an extract of *Euphorbia prostrata* with long-term effectiveness and low prolapse rates. The treatment includes administration by oral route an effective amount of composition comprising of a pharmaceutically acceptable carrier and mixture of flavonoids and phenolic compounds extracted from *Euphorbia prostrata*. The treatment also includes local application to the hemorrhoids and anorectal tissues, an effective amount of composition comprising of a pharmaceutically acceptable carrier and a mixture of flavonoids and phenolic compounds extracted from *Euphorbia prostrata*.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compositions that can be orally administered as well as uniformly applied to the affected region. It reduces inflammation, and soothes the feeling of itching and burning associated with it. The invention provides relief from pain associated with hemorrhoids. The invention also significantly reduces bleeding and accelerates tissue re-growth in the affected hemorrhoidal tissue. The invention is useful in the treatment of lesions, other than hemorrhoids in the anorectal area and can be formulated in several types of dosage forms. There are no side effects from the use of the composition in human beings. Further, the treatment is not physically or psychologically unpleasant in its administration and/or application. The plant *Euphorbia prostrata* (Family: Euphorbiaceae) was identified as being relevant in the study of anorectal and colonic diseases, including hemorrhoids. *Euphorbia prostrata* is well known to the Indian traditional medicine in the use of treatment for asthma, bloody dysentery and sores. Previously, five new compounds were discovered and identified by the inventors in *Euphorbia prostrata* namely luteolin, 6-methoxy-quercetin-glycoside, Quercetin, and glycosides of luteolin and apigenin. Now two more phenolic compounds, namely ellagic acid and gallic acid were identified in the extract prepared in a different manner, which were found to be of additional therapeutic value for treatment of hemorrhoids.

The compounds of the present invention are standardized to pharmaceutically acceptable specifications in order to ensure reproducibility from batch to batch. The result is the extract of *Euphorbia prostrata*, which is the main active agent in the improved anorectal composition. Another unique feature of this extract of *Euphorbia prostrata* is that it is prepared in such a manner that the resulting composition is easily dispersible in water due to presence of many hydrophilic compounds besides flavonoids.

The pharmaceutical composition comprising of the extract of *Euphorbia prostrata* as the active ingredient contains flavonoids and phenolic compounds, out of which apigenin-7-glycoside is about 1 - 4% by weight of the extract, luteolin-7-glycoside is about 0.3 - 2% by weight of the extract, and apigenin, luteolin and quercetin are about 0.001 – 0.3% by weight of the extract. The extract also contains 1 - 10% by weight of tannins, 1-15% by weight of ellagic acid, and 1 – 12% by weight of gallic acid.

In an embodiment of the present invention, *Euphorbia prostrata* was found to be devoid of any toxic diterpene content like phorbol or ingenol esters, unlike many other species of *Euphorbia*.

The pharmaceutical compositions of the present invention may also contain additional therapeutic agents from other plants and/or from different pharmacological groups such as anesthetics, vasoconstrictors, protectants, counterirritants, astringents, wound healing agents, antimicrobials, keratolytics, anticholinergics or their pharmaceutically acceptable salts, used either alone or in combinations thereof.

Preferably, it would be beneficial to include other wound healing and antimicrobial agents, which will result in the improvement of the effectiveness of the composition.

The anesthetics include but are not limited to benzocaine, diperodon, pramoxine, camphor, dibucaine, phenol, tetrtacaine, lignocaine and phenacaine, used either alone or in combinations thereof. The amount of such anesthetics could vary between 0.25% and 25% by weight.

The vasoconstrictors include but are not limited to ephedrine and phenylephrine, used either alone or in combinations thereof. The amount of such vasoconstrictors may vary between 0.005% and 1.5% by weight.

The protectants include but are not limited to aluminum hydroxide gel, calamine, cocoa butter, cod or shark liver oil, glycerin in aqueous solution, kaolin, lanolin, mineral oil, starch, white petrolatum, wool alcohol, zinc oxide, vegetable or castor oil, polyethylene glycol and propylene glycol, used either alone or in combinations thereof. The amount of such protectants may vary between 5.0% and 88.0% by weight.

The counterirritant includes but is not limited to menthol in aqueous solution. The amount of such counterirritant may vary between 0.25 - 2.5% by weight.

The astringents include but are not limited to calamine, zinc oxide, hamamelis water, bismuthresorcinol compound, bismuth subgallate, Peruvian balsam, aluminium chlorhydroxy allantoinate, tannic acid and tannins, used either alone or in combinations thereof. The amount of such astringents may vary between 0.2% and 60.0% by weight. The tannins additionally may be derived from plants such as *Butea monosperma, Butea parviflora* and *Butea frondoza* (Family: Leguminosae).

The wound healing agents include but are not limited to vitamin A and vitamin D in an amount of between 0.005% and 0.04% by weight. Also Peruvian balsam can be included by weight in an amount of between 0.5% and 2.5% by weight. Also cod liver oil can be included in an amount between 1.0% and 6.0% by weight.

The antimicrobial agents include but are not limited to benzethonium chloride, benzalkonium chloride, boric acid, 8-quinolinoi benzoate, secondary amyltricresols, cetylpyridinium chloride, phenol, menthol, chlorothymol, camphor and 8-hydroxyquinoline sulfate, used either alone or in combinations thereof. The amount of such antimicrobial agents may vary between 0.02% and 40.0% by weight.

The keratolytics include but are not limited to aluminium chlorhydroxy allantoinate and resorcinol, used either alone or in combinations thereof. The amount of such keratolytics may vary between 0.2% and 3.5% by weight.

The anticholinergics include but are not limited to atropine or other solanaceous type alkaloids, used either alone or in combination thereof. The amount of such anticholinergics may vary between 0.02% and 0.1% by weight.

The pharmaceutical compositions of the present invention can be prepared by dissolving or dispersing the extract in appropriate base(s)/carrier(s). The pharmaceutical composition into different dosage forms can be formulated using conventional excipients and techniques known to art. Pharmaceutical dosage bases or carriers used in the present invention can be capsules (hard or soft), tablets (coated or uncoated), ointments, creams, gels, foams, solutions, suspensions, medicated pad, bandage, powder, aerosols, sprays, film, flakes, modified release dosage forms (sustained release, controlled release, delayed release, prolonged release, timed release, and the like) sublingual dosage forms, wafers, caplets, parenteral dosage forms to be infiltered at the site of the injection, and the like.

The capsules comprise of 25–300 mg of the extract of *Euphorbia prostrata*, preferably 50–100 mg along with pharmaceutical excipients. Similarly, tablets may be prepared by dispersing 25–300 mg of the extract of *Euphorbia prostrata*, preferably 50-100 mg in a suitable carrier, optionally along with other pharmaceutical excipients. The tablets may be coated or uncoated.

The cream or ointment may contain 0.1 - 10% w/w, preferably 0.2 - 5% w/w of the extract of *Euphorbia prostrata*.

In an embodiment of the present invention, the capsule may be taken, subject to a maximum of 300 mg of extract per day, along with topical application containing the same extract, as and when required.

The granules in ready dispersible and effervescent form may be prepared by using excipients such as sucrose, mannitol, sodium bicarbonate, citric acid, and the like.

The cream may be prepared by emulsifying the aqueous phase, containing 0.1 - 10% w/w preferably 0.2 - 5% w/w of the extract, along with a suitable oleaginous phase.

Other alternatives can be prepared by formulating the extract in 0.1 - 10% w/w as Hydrophilic ointment USP with absorption bases; or water soluble bases such as Polyethylene glycol ointment USNF; or as water absorbing bases such as Hydrophilic petrolatum USP, Lanolin USP; or in hydrocarbon bases such as White petrolatum USP.

The suppository compositions may contain either hydrophobic or hydrophilic base and includes cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights, polyoxyethylene sorbitan fatty acid esters and polyethylene stearates, polyvinyl alcohol, polyvinyl pyrrolidone, polyacrylamide, chemically modified starch or a combination of these materials.

The foam and spray bases may contain one or more of aqueous and nonaqueous solvents, propellants, surfactants, suspending agents and stabilizing agents.

The medicated pads may contain one or more of the following: Water, glycerin, propylene glycol, alcohol and Hamamelis water.

EVALUATION OF PHARMACOLOGICAL ACTIVITY OF THE EXTRACT

Antihaemorrhoidal activity

The antihaemorrhoidal activity of the *Euphorbia prostrata* extract was assessed and compared with reference drug, diosmin using anorectal : body weight ratio model in rats (modified method of Jia *et al.*, 2000). *Euphorbia prostrata* extract (5, 10, 20 and 40 mg/kg, p.o., 7 days) showed significant decrease in anorectal : bodyweight ratio as well as inflammation and redness at the site when compared to control (carrageenan - treated group). Diosmin (50 mg/kg, p.o., 7 days) also significantly decreased anorectal : body weight ratio in rats. Further, individual components of *Euphorbia prostrata* extract i.e. apigenin-7- glucoside (0.478 mg/kg, p.o.), ellagic acid (1.204 mg/kg, p.o.), and gallic acid (1.123 mg/kg, p.o.) on chronic administration (7 days) also showed effect. In histopathological examination of the anorectum, normal animals administered per orally with 0.5% CMC alone showed intact mucosal layer with prominent mucosal cells and mild leukocyte migration, where as carrageenan - treated animals showed

uneven thick mucosal layer with disrupted mucosal cells and severe leukocyte migration suggesting the presence of a significant inflammation. Further, both *Euphorbia prostrata* extract (10 mg/kg, p.o., 7 days) and diosmin (50mg/kg, p.o., 7 days) showed intact mucosal layer with prominent mucosal cells and mild leukocyte migration.

Anti-inflammatory activity

Carrageenan – induced paw edema in rats

Carrageenan (1% w/v) produced paw edema (Winter *et al.*, 1962) in control group, indicating inflammatory response. *Euphorbia prostrata* extract (5, 10, 20, and 40 mg/kg, p.o.) dose dependently and significantly decreased carrageenan-induced increase in paw volume as compared to control rats (ED_{50} 15.42 – 15.84 mg/kg, p.o.) The onset of anti-inflammatory effect was rapid and lasted up to 4 hrs after carrageenan injection. The anti-inflammatory effect of *Euphorbia prostrata* extract at doses 20 and 40 mg/kg was comparable to that of nimesulide (2 mg/kg, p.o.), a preferential cyclooxygenase – 2 (COX – 2) inhibiting NSAID.

Further, solution of *Euphorbia prostrata* extract (0.5- 4.0 % equivalent to 1 – 8 mg/kg, applied topically on paw) significantly decreased the carrageenan – induced increase in paw volume. The topical anti-inflammatory effect of *Euphorbia prostrata* extract (1%) was comparable to that of nimesulide (2%) at 60 min in rats.

Antinociceptive activity

Acetic acid – induced writhing in mice (Koster *et al.*, 1959)

Euphorbia prostrata extract (2 mg/kg, p.o.) exhibited maximum antinociceptive effect after 90 minutes of its administration in time response study. This effect lasted up to 2 hrs of its administration. Nimesulide (2 mg/kg), a reference drug also significantly increased the pain threshold in mice. *Euphorbia prostrata* extract (1, 2, 5, and 10 mg/kg, p.o.) produced dose - dependent antinociceptive effect in mice. Apigenin -7- glucoside (0.5, 1.0, and 2.0 mg/kg, p.o.) and luteolin glucoside (0.25, 0.5, and 1.0 mg/kg, p.o.) also exhibited dose-dependent antinociceptive effect in writhing test.

Carrageenan – induced hyperalgesia in rats

Carrageenan (1% w/v) significantly decreased paw withdrawal latency in paw pressure test (Randall and Selitto, 1957). *Euphorbia prostrata* extract (10, 20, and 40 mg/kg, p.o.)

significantly increased paw withdrawal latency as compared to carrageenan - treated rats. Nimesulide (2 mg/kg), a reference drug also increased paw withdrawal latencies in rats.

Carrageenan – induced pleurisy in rats

Pleurisy was induced with carrageenan using a method reported by Engelhard *et al.*, 1995. A single-dose administration of *Euphorbia prostrata* extract (20 and 40 mg/kg, p.o.) produced significant inhibition of exudates formation and migration of polymorphonuclear leukocytes and monocytes in carrageenan-induced pleurisy.

Superoxide radical scavenging activity

Euphorbia prostrata extract (25 µg) exhibited a superior superoxide radical scavenging activity of 38.70% in comparison to tocopherol (25 µg), which showed a similar activity of 25.54%.

Wound healing activity in rats

The wounds were developed by skin excision method in rats as described by Vishnu Rao *et al.*, 1996. *Euphorbia prostrata* extract cream (1.75%) showed significant wound healing activity in comparison to placebo cream on Day 4, 8, and 12 in wound excision model in rats.

Haemostatic activity

Euphorbia prostrata extract (1%, 2%, and 4% solution) significantly reduced the bleeding time as compared to control group (distilled water). Moreover, the reduction in bleeding time with 4% solution of *Euphorbia prostrata* extract was comparable to 2.5% solution of alginic acid.

MECHANISM OF ANTI-HEMORRHOIDAL ACTIVITY OF THE EXTRACT:

Euphorbia prostrata extract mainly comprises of flavonoids (apigenin -7- glucoside, luteolin -7 –glucoside), ellagic acid, gallic acid, and tannins. Phenolic compounds are widespread in the plant kingdom. The major groups of phenolic compounds are flavonoids and phenolic acids. They are one of the main constituents of several medicinal plants that have been used as folk medicine throughout the world. Interest has recently been focused on flavonoids and flavanoids because of their broad pharmacological activities. These flavonoids are well reported for analgesic, anti-inflammatory, antioxidant, antiangiogenic, anti–allergic, antiviral and antimutagenic activity (Lin et al., 2001; Formica and Regelson, 1995; Fotsis et al., 1997; Wang

et al., 1998; Block et al., 1998). It is reported that apigenin is a most potent inhibitor of transcriptional activation of both COX-2 and iNOS enzyme in lipopolysaccharide activated RAW 264.7 macrophages. It is further suggested that suppression of transcriptional activation of COX-2 and iNOS by apigenin might mainly be mediated through inhibition of IκB (inhibitor of κB). Such type of modulation of COX-2 and iNOS by apigenin may be important in the prevention of carcinogenesis and inflammation (Liang et al., 1999). Further, it is also suggested that antioxidant properties of apigenin -7 – glucoside contribute to its anti-inflammatory activity in various animal models (Fuchs and Milbradt, 1993). Della Loggia *et al.*, 1986 also reported that apigenin -7-glucoside and luteolin -7 -galactoside shows a dose dependent inhibition of the oedematous response to croton oil. In CCl_4 – induced peroxidation, apigenin and luteolin had shown significant antiperoxidative activity in rat liver microsomes (Cholbi et al., 1991). Xagorari et al., 2001 reported that luteolin inhibits protein tyrosin phosphorylation, nuclear factor- κB mediated gene expression and pro-inflammatory cytokine production in murine macrophages. Ellagic acid is one of the major constituents of *Euphorbia prostrata* extract also reported to suppress histamine release mediated by histamine liberators (compound 48/80, dextran and polymyxin B sulfate) *in vivo* (Bhargava and Westfall, 1969). Moreover, the anti-inflammatory effects of flavonoids comprise inhibition of histamine release, modulation of the prostanoids metabolism and antioxidant properties. It is speculated that analgesic, anti-inflammatory and antioxidant activity of various flavonoid components of *Euphorbia prostrata* extract (apigenin-7-glucoside, luteolin -7 -glucoside) and ellagic acid and/or gallic acid may contribute in healing of inflammatory tissue damage in hemorrhoidal conditions.

Phenolic acids are reported to activate intrinsic blood coagulation by activation of Hageman factor and cause a state of hypercoagulability. Although the hypercoagulable state persists for as long as 4 hours after i.v. administration, no thrombotic phenomena has been reported (Girolami and Cliffton, 1967).

Vegetable tannins are water-soluble phenolic compounds including both hydrolysable and condensed tannins that are present in every food plant. Hydrolysable tannins contain either gallotannins or ellagiotannins that yield gallic acid or ellagic acid respectively on hydrolysis. It is well reported that tannic acid has antimicrobial properties, which is associated with the ester linkage between gallic acid and other sugar or alcohol groups (Chung *et al.*, 1993; 1995).

From the anti-hemorrhoidal studies conducted in animals, it is evident that the *Euphorbia prostrata* extract has better efficacy than the purified flavonoids or other constituents alone.

The various studies conducted on *Euphorbia prostrata* extract are listed below in Figures 1-9.

Figure 1: Effect of *Euphorbia prostrata* extract (14395) against carrageenan-induced hemorrhoids in rats.

Figure 2: Effect of individual component of *Euphorbia prostrata* extract (14395) against carrageenan-induced hemorrhoids in rats.

Figure 3: Effect of *Euphorbia prostrata* extract (14395) against carrageenan-induced paw oedema (oral).

Figure 4: Effect of *Euphorbia prostrata* extract (14395) against carrageenan-induced paw oedema (topical).

Figure 5: Effect of *Euphorbia prostrata* extract (14395) against acetic acid-induced chemonociception in mice.

Figure 6: Effect of principal components of *Euphorbia prostrata* extract (14395) against acetic acid-induced chemonociception in mice.

Figure 7: Effect of *Euphorbia prostrata* extract (14395) against carageenan-induced hyperalgesia in rats.

Figure 8: Effect of *Euphorbia prostrata* extract (14395) on bleeding time in liver incision model.

Figure 9: *In vitro* superoxide radical scavenging activity of *Euphorbia prostrata* extract (14395).

SAFETY STUDIES

Effect on central nervous system

The effect of *Euphorbia prostrata* extract on the central nervous system were assessed from acute studies of effect on the appearance and gross behavior of rats and mice, the performance of mice on a rotating rod, open field behavior in rats, locomotor activity in mice using actophotometer, rectal temperature in rats, forced swimming despair behavior in mice, pentobarbitone-induced sleeping time in mice. Behaviorally, *Euphorbia prostrata* extract was well tolerated by both mice and rats (up to 2000 mg/kg, p.o.) following single oral administration and following multiple dose oral administration (up to 130 mg/kg in mice for 28 days and up to

90 mg/kg in rats for 28 days). In diazepam-controlled mouse studies, *Euphorbia prostrata* extract did not (a) alter gross behavior; (b) impair motor co-ordination (rotarod test); (c) impair motor activity using actophotometer after oral administration at doses 100, 200 and 400 mg/kg. In chlorpromazine-controlled rat study, *Euphorbia prostrata* extract did not alter rectal temperature at doses 100, 200, and 400 mg/kg. In imipramine-controlled mouse study, *Euphorbia prostrata* extract at doses 100, 200, and 400 mg/kg did not alter forced swimming despair behavior after single oral administration. Furthermore, *Euphorbia prostrata* extract did not interact with pentobarbitone-induced sleeping time at doses 100, 200, and 400 mg/kg in mice. In diazepam-controlled rat study, *Euphorbia prostrata* extract did not impair open field behavior (ambulatory and rearing behavior) at doses 100, 200, and 400 mg/kg.

Effect on cardiovascular system

Euphorbia prostrata extract did not cause any change in normal ECG, blood pressure, and heart rate in rats following single oral administration up to 400 mg/kg and following multiple dose oral administration (up to 90 mg/kg in rats for 28 days).

Effect of on respiratory system

Euphorbia prostrata extract did not alter basal insufflation pressure of trachea after 7 days administration of 400 mg/kg, p.o. of *Euphorbia prostrata* extract in guinea pig.

Effect on gastrointestinal system

Euphorbia prostrata extract did not alter basal acid secretion and gastrointestinal integrity upon single administration up to 400 mg/kg, p. o. in rats. In mice, there was no alteration in guf motility up to 400 mg/kg, p.o. of *Euphorbia prostrata* extract after 1 h of single dose oral administration.

TOXICOLOGICAL STUDIES

Single dose toxicity:

No mortality was observed when *Euphorbia prostrata* extract was administered up to 2000 mg/kg, p.o. in rats and mice.

Repeat dose toxicity:

Mice: Repeated administration (32.50 mg/kg, 65.0 mg/kg and 130.0 mg/kg, p.o.) of *Euphorbia prostrata* extract for 28 days to mice did not exhibit mortality (NOEL 130 mg/kg, p.o. in mice).

Rats: Repeated administration (22.50 mg/kg, 45.0 mg/kg and 90.0 mg/kg, p.o.) of *Euphorbia prostrata* extract for 28 days to rats did not exhibit mortality (NOEL 90 mg/kg, p.o.). In another study, repeated administration of *Euphorbia prostrata* extract 428 mg/kg, p.o.. for 14 days did not produce significant alterations in body weight, organ weights, biochemical, and histopathological changes in comparison to control animals.

Guinea pig: Repeated administration of *Euphorbia prostrata* extract up to 300 mg/kg to guinea pig for 14 days did not produce significant alterations in body weight, organ weights, biochemical, and histopathological changes in comparison to control animals.

Examples are provided below to illustrate the aspects of the present invention. However, they do not intend to limit the scope of the present invention.

EXAMPLES

General process of manufacture of the Extract:

Qualified professionals collected the plant *Euphorbia prostrata* from various parts of India. The plant was identified and characterized according to WHO guidelines (WHO/TRM/91.4, Programme Traditional Medicines World Health Organization Geneva, 1991) and was dried under controlled conditions of temperature and humidity. The whole plant was ground to coarse powder. The coarse powder was extracted using a polar solvent like an alkanol or acetone with or without water. The extract was concentrated and washed with a non-polar solvent like a hydrocarbon or chlorinated hydrocarbon. The washed extract was optionally further extracted into a medium polar solvent like ethyl acetate or ethyl methyl ketone. The final extract was optionally dehydrated with a suitable dehydrating agent and dried, either in a tray drier or in a spray drier, milled to a powder form, sifted to the desired particle size and packed in a suitable container to protect from moisture.

EXAMPLE 1

The powdered drug (500 kg) was packed in a S.S. extractor. The extraction was affected by percolation with 3000 lt. of 80% aqueous methanol at about 60°C. The process was repeated 5 times till the drug was exhausted. The aqueous-methanolic extracts were combined and concentrated by distillation. The concentrated extract was washed with 5-10 volumes of hexane to remove the wax and fatty material. The washed extract was dried completely for several hours at 60°C under vacuum. The final extract was milled to a fine powder, sifted for uniform particle size and packed to protect from the moisture.

EXAMPLE 2

The powdered drug (700 kg) was packed in a S.S. extractor. The extraction was affected by percolation with 4500 lt. of methanol at about 60°C. The process was repeated 5 times till the drug was exhausted. The methanolic extracts were combined and concentrated by distillation. The concentrated extract was washed with 5-10 volumes of dichloromethane to remove the wax and fatty material. The washed extract was dried completely for several hours at 60°C under vacuum. The final extract was milled to a fine powder, sifted for uniform particle size and packed to protect from the moisture..

EXAMPLE 3

The powdered drug (350 kg) was packed in a S.S. extractor. The extraction was affected by percolation with 3000 lt. of 80% aqueous acetone at about 50°C. The process was repeated 5 times till the drug was exhausted. The aqueous-acetone extracts were combined and concentrated by distillation. The concentrated extract was washed with 5-10 volumes of hexane to remove the wax and fatty material. The washed extract was dried completely for several hours at 60°C under vacuum. The final extract was milled to a fine powder, sifted for uniform particle size and packed to protect from the moisture.

EXAMPLE 4

The powdered drug (500 kg) was packed in a S.S. extractor. The extraction was affected by percolation with 3000 lt. of 80% aqueous methanol at about 60°C. The process was repeated 5 times till the drug was exhausted. The aqueous-methanolic extracts were combined and concentrated by distillation. The concentrated extract was washed with 5-10 volumes of hexane to remove the wax and fatty material. The washed extract was again extracted with ethyl acetate. The ethyl acetate extract was dehydrated with anhydrous sodium sulphate and concentrated by distillation. The concentrated extract was dried completely for several hours at 60°C under vacuum. The final purified extract was milled to a fine powder, sifted for uniform particle size and packed to protect from the moisture.

Process of evaluation of the Extract

The extract of *Euphorbia prostrata* of the above-mentioned examples was characterised by High Performance Liquid Chromatography (HPLC). The HPLC was performed under following conditions and using Waters system equipped with M510 pumps and data station with Millenium software.

Mobile phase: A linear gradient of Mobile Phase A (2% acetic acid in Water) and Mobile Phase B (2% acetic acid in Acetonitrile) according to the following table:

Time (minutes)	Mobile Phase 'A'%	Mobile Phase 'B'%	Comments
0	90	10	Equilibration
0 - 2	90	10	Isocratic
2 - 30	65	35	Linear Gradient
30 - 35	65	35	Isocratic
35 - 40	90	10	Linear Gradient
40 - 45	90	10	Isocratic

Column: C_{18} (250X4.6 mm/5 μ)

Flow Rate: 1 ml/min

Detector: UV absorbance at 335 nm

The HPLC chromatogram showed a number of peaks, the major ones corresponding to gallic acid, ellagic acid, luteolin glucoside and apigenin glucoside. The two peaks corresponding to the flavonoid components luteolin glucoside and apigenin glucoside were used as the chemical and pharmacological marker for quantitation of the product. A sum of the two peaks was calculated corresponding to standard apigenin glucoside and the measure was expressed as the Total Flavonoids.

CAPSULE COMPOSITIONS

EXAMPLE 5

Ingredient	**mg/capsule**
Purified euphorbia extract	100.0
Microcrystalline cellulose	200.8
Mannitol	72.0
Talc	3.2

Sodium starch glycollate	12.0
Colloidal silicon dioxide	12.0

Procedure:

1) Extract, microcrystalline cellulose and mannitol are sifted and mixed together.
2) Talc, sodium starch glycollate and colloidal silicon dioxide are passed through fine sieves individually and then mixed together.
3) The materials of step 1 and 2 are mixed.
4) The material of step 3 is filled into empty hard gelatin capsules at an average fill weight of 400 mg ± 2%.
5) The filled capsules are packed in air-tight packages.

EXAMPLE 6

Ingredient	**mg/capsule**
Purified euphorbia extract	100.0
Microcrystalline cellulose	150.0
Mannitol	65.0
Lactose	50.0
Talc	3.0
Sodium starch glycollate	17.0
Colloidal silicon dioxide	15.0

Procedure:

1) Extract, microcrystalline cellulose, lactose and mannitol are sifted and mixed together.
2) Talc, sodium starch glycollate and colloidal silicon dioxide are passed through fine sieves individually and then mixed together.
3) The materials of step 1 and 2 are mixed.
4) The material of step 3 is filled into empty hard gelatin capsules at an average fill weight of 400 mg ± 2%.
5) The filled capsules are packed in air-tight packages.

EXAMPLE 7

Ingredient	**mg/capsule**
Purified euphorbia extract	100.0
Microcrystalline cellulose	50.0

Mannitol	65.0
Lactose	150.0
Talc	3.0
Sodium starch glycollate	17.0
Colloidal silicon dioxide	15.0

Procedure:

1) Extract, microcrystalline cellulose, lactose and mannitol are sifted and mixed together.
2) Talc, sodium starch glycollate and colloidal silicon dioxide are passed through fine sieves individually and then mixed together.
3) The materials of step 1 and 2 are mixed.
4) The material of step 3 is filled into empty hard gelatin capsules at an average fill weight of 400 mg ± 2%.
5) The filled capsules are packed in air-tight packages.

EXAMPLE 8

Ingredient	**mg/capsule**
Purified euphorbia extract	100.0
Microcrystalline cellulose	175.0
Mannitol	80.0
Talc	5.0
Sodium starch glycollate	15.0
Colloidal silicon dioxide	25.0

Procedure:

1) Extract, microcrystalline cellulose and mannitol are sifted and mixed together.
2) Talc, sodium starch glycollate and colloidal silicon dioxide are passed through fine sieves individually and then mixed together.
3) The materials of step 1 and 2 are mixed.
4) The material of step 3 is filled into empty hard gelatin capsules at an average fill weight of 400 mg ± 2%.
5) The filled capsules are packed in air-tight packages.

EXAMPLE 9

Ingredient	**mg/capsule**

Purified euphorbia extract	100.0
Microcrystalline cellulose	135.0
Starch	25.0
Dibasic calcium phosphate	110.0
Talc	2.0
Magnesium stearate	3.0
Sodium starch glycollate	10.0
Colloidal silicon dioxide	15.0

Procedure:

1) Extract, microcrystalline cellulose, starch and dibasic calcium phosphate are sifted and mixed well.
2) Talc, magnesium stearate, sodium starch glycollate and colloidal silicon dioxide are passed through fine sieves individually and then mixed together.
3) The materials of step 1 and 2 are mixed.
4) The material of step 3 is filled into empty hard gelatin capsules at an average fill weight of 400 mg ± 2%.
5) The filled capsules are packed in air-tight packages.

EXAMPLE 10

Ingredient	**mg/capsule**
Purified euphorbia extract	100.0
Microcrystalline cellulose	90.0
Lactose	50.0
Starch	122.0
Talc	3.0
Magnesium stearate	3.0
Croscarmellose sodium	12.0
Colloidal silicon dioxide	20.0

Procedure:

1) Extract, microcrystalline cellulose, lactose & starch are sifted and mixed together.
2) Talc, magnesium stearate, croscarmellose sodium and colloidal silicon dioxide are passed through fine sieves individually and then mixed together.

3) The materials of step 1 and 2 are mixed.

4) The material of step 3 is filled into empty hard gelatin capsules at an average fill weight of 400 mg ± 2%.

5) The filled capsules are packed in air-tight packages.

EXAMPLE 11

Ingredient	**mg/capsule**
Purified euphorbia extract	100.0
Mannitol	176.0
Starch	100.0
Talc	2.0
Croscarmellose sodium	5.0
Sodium starch glycollate	12.0
Sodium stearyl fumarate	5.0

Procedure:

1) Extract, mannitol and starch are sifted and mixed together.

2) Talc, croscarmellose sodium, sodium starch glycollate and sodium stearyl furmate are passed through fine sieves individually and then mixed together.

3) The materials of step 1 and 2 are mixed.

4) The material of step 3 is filled into empty hard gelatin capsules at an average fill weight of 400 mg ± 2%.

5) The filled capsules are packed in air-tight packages.

TABLET COMPOSITIONS

EXAMPLE 12 (Uncoated tablet)

Ingredient	**mg/tablet**
Purified euphorbia extract	100.0
Microcrystalline cellulose	120.0
Mannitol	80.0
Croscarmellose sodium	10.0
Lactose	66.0
Talc	4.0
Colloidal silicon dioxide	10.0

Croscarmellose sodium	10.0

Procedure:

1) Extract, microcrystalline cellulose, mannitol, croscarmellose sodium and lactose are sifted and mixed together.
2) The material of step 1 is compacted.
3) The compacts of step 2 are passed through sieve and mixed.
4) Talc, colloidal silicon dioxide and croscarmellose sodium are passed through fine sieve and mixed together.
5) The material of step 3 is mixed with material of step 4.
6) The material of step 5 is compressed into tablets at an average weight of 400 mg ± 2%.
7) The tablets are packed in air-tight packages.

EXAMPLE 13 (Film-coated tablet)

Ingredient	**mg/tablet**
Core tablet composition	
Purified euphorbia extract	100.0
Microcrystalline cellulose	120.0
Mannitol	80.0
Croscarmellose sodium	10.0
Lactose	66.0
Talc	4.0
Colloidal silicon dioxide	10.0
Croscarmellose sodium	10.0
Film coating composition	
Hydroxypropyl methylcellulose (E-15)	12.0
Polyethylene glycol 400 (PEG 400)	2.4
Iron oxide red	0.75
Iron oxide yellow	0.50
Titanium dioxide	0.25
Isopropyl alcohol	q.s. (lost in processing)
Dichloromethane	q.s. (lost in processing)

Procedure:

1) Extract, microcrystalline cellulose, mannitol, croscarmellose sodium and lactose are sifted and mixed together.
2) The material of step 1 is compacted.
3) The compacts of step 2 are passed through sieve and mixed.
4) Talc, colloidal silicon dioxide and croscarmellose sodium are passed through fine sieve and mixed together.
5) The material of step 3 is mixed with material of step 4.
6) The material of step 5 is compressed into tablets at an average weight of 400 mg ± 2%.
7) Hydroxypropyl methylcellulose is dispersed in a mixture of isopropyl alcohol and dichloromethane with continuous mixing in homogenizer.
8) PEG 400 is added to the above solution of step 7 and mixed.
9) Iron oxide red, iron oxide yellow and titanium dioxide are passed through fine sieve and mixed.
10) The material of step 9 is added to material of step 8 and mixed for 30 minutes.
11) The core tablets are charged into the coating pan and coated with the coating solution of step 10 till an average tablet weight gain of ~3% is achieved.
12) The tablets are dried and packed in air-tight packages.

CREAM COMPOSITIONS

EXAMPLE 14

Ingredient	**mg/gm**
Purified euphorbia extract	10.0
Propylene glycol	50.0
Titanium dioxide	10.0
Stearic acid	130.0
Cetyl alcohol	10.0
Isopropyl myristate	60.0
Sorbitan stearate	20.0

Methyl paraben	1.5
Propyl paraben	0.3
Corn oil	50.0
Glycerin	50.0
Sorbitol solution	30.0
Veegum HV	10.0
Sodium CMC	3.0
Tween 80	15.0
Purified water	9.8

Procedure:

1) Purified Euphorbia extract, methyl paraben and propyl paraben are dissolved in propylene glycol; the mixture heated to 55-60^{0}C; titanium dioxide is added to it and stirred well.
2) Stearic acid, cetyl alcohol, isopropyl myristate, sorbitan stearate, and corn oil are heated to 70^0-75^{0}C.
3) In another vessel, sorbitol solution and Tween 80 are taken.
4) Veegum HV is separately hydrated in the water.
5) Sodium carboxymethyl cellulose (sodium CMC) is separately hydrated in glycerin.
6) The material of step 4 and step 5 are added to the material of step 3 and heated to 70^0-75^{0}C.
7) The material of step 2 and step 6 are mixed and cooled.
8) When the material of step 7 attains a temperature of 50^0-55^{0}C, the material of step 1 is added to it.
9) The mixture is allowed to cool to room temperature to obtain the cream.

EXAMPLE 15

Ingredient	**mg/gm**
Purified euphorbia extract	10.0
Propylene glycol	50.0
Titanium dioxide	10.0
Hard paraffin	60.0
Liquid paraffin	10.0

Isopropyl myristate	30.0
Span 60	20.0
Methyl paraben	1.5
Propyl paraben	0.3
Corn Oil	20.0
Glycerin	80.0
Sorbitol solution	50.0
Veegum HV	20.0
Tween 80	15.0
Purified water	9.8

Procedure:

1. Purified Euphorbia extract, methyl paraben and propyl paraben are dissolved in propylene glycol; the mixture heated to 55-60^{0}C; titanium dioxide is added to it and stirred well.
2. Hard paraffin, liquid paraffin, isopropyl myristate, Span 60, and Corn Oil are heated to 70^0-75^{0}C.
3. Veegum HV is hydrated in purified water; glycerin, Tween 80, and sorbitol is added to it; and the mixture is heated to 70^0-75^{0}C.
4. The material of step 2 is added to the material of step 3 with stirring and the mixture is allowed to cool to 55^0-60^{0}C.
5. The material of step 1 is added to the material of step 4, stirred, and allowed to cool to room temperature to obtain the cream.

EXAMPLE 16

Ingredient	**mg/gm**
Purified euphorbia extract	10.0
Propylene glycol	50.0
Titanium dioxide	10.0
Glyceryl monostearate	90.0
Hydrogenated lanolin	30.0
Corn oil	40.0
Simethicone	1.5

Span 60	20.0
Purified water	9.8
Hydroxyethyl cellulose	20.0
Glycerin	50.0
Sorbitol	30.0
Sodium CMC	1.5
Propyl paraben	0.3
Methyl paraben	1.5
Tween 80	15.0
Purified water	9.8

Procedure:

1. Purified Euphorbia extract, methyl paraben and propyl paraben are dissolved in propylene glycol; titanium dioxide is added to it and stirred well.
2. Glyceryl monostearate, hydrogenated lanolin, corn oil, simethicane, and Span 60 are taken.
3. In cool purified water, hydroxyethyl cellulose is dissolved; sorbitol and Tween 80 is added to it and the mixture is heated to 70-75^{0}C.
4. Separately sodium carboxymethyl cellulose (sodium CMC) is dispersed in glycerin and added to the material of step 3.
5. The material of step 2 is added to the material of step 3 and allowed to cool with stirring.
6. When a temperature of 50-55^{0}C is attained, the material of step 1 is added, stirred, and allowed to cool to room temperature to obtain the cream.

EXAMPLE 17

Ingredient	**mg/gm**
Purified euphorbia extract	10.0
Beeswax	50.0
Liquid paraffin	60.0
Corn oil	25.0
Stearic acid	110.0
Cetyl alcohol	10.0

Titanium dioxide	10.0
Propylene glycol	50.0
Methyl paraben	1.5
Propyl paraben	0.3
Glycerin	50.0
Sorbitol Solution	30.0
Tween 80	15.0
Purified water	9.8

Procedure:

1. Purified Euphorbia extract, methyl paraben and propyl paraben are dissolved in propylene glycol; titanium dioxide is added to it and stirred well.
2. Beeswax, liquid paraffin, corn oil, stearic acid and cetyl alcohol are heated to 70-75^{0}C.
3. Glycerin, sorbitol and Tween 80 is added to purified water and heated to 70^0-75^{0}C.
4. The material of step 2 is added to the material of step 3 and stirred.
5. The material of step 1 is added to the material of step 4 and allowed to cool to room temperature to obtain the cream.

EXAMPLE 18

Ingredient	**mg/gm**
Purified euphorbia extract	10.0
Propylene glycol	50.0
Titanium dioxide	10.0
Stearic acid	70.0
Simethicone	1.0
Glyceryl monostearate	60.0
Cetosteryl alcohol	20.0
Cetyl alcohol	10.0
Sorbitan stearate	20.0
Methyl paraben	1.5
Propyl paraben	0.3
Glycerin	50.0

Sorbitol	30.0
Tween 80	15.0
Xanthan gum	10.0
Purified water	9.8

Procedure:

1. Purified Euphorbia extract, methyl paraben and propyl paraben are dissolved in propylene glycol; titanium dioxide is added to it and stirred well.
2. Stearic acid, simethicone, glyceryl monostearate, cetosteryl alcohol, cetyl alcohol, and sorbitan stearate are heated to 70^0-75^0C.
3. Glycerin, sorbitol, Tween 80 and purified water are heated to 70^0-75^0C.
4. Xanthum gum is dispersed in glycerin and added to the material of step 3.
5. The material of step 2 is added to the material of step 4 and allowed to cool.
6. The material of step 1 is added to the material of step 5 and allowed to cool to room temperature to obtain the cream.

SUPPOSITORY COMPOSITIONS

EXAMPLE 19

Ingredient	**gm/10 units**
Purified euphorbia extract	0.50
Polyethylene glycol 4000-(PEG 4000)	3.56
Polyethylene glycol 1000 (PEG 1000)	12.46
Polyethylene glycol 400 (PEG 400)	1.78
Propylene glycol	1.50
Glycerin	0.20

Procedure:

1) PEG 4000, PEG 1000 and PEG 400 are melted together and mixed well.
2) Euphorbia extract is dissolved in propylene glycol at 40-45^{0}C with constant stirring.
3) The material of step 2 is added to the material of step 1 and mixed well.
4) The material of step 3 is poured into suppository moulds and cooled.
5) Suppositories thus formed are removed from moulds and packed suitably.

EXAMPLE 20

Ingredient	**gm/10 units**
Purified euphorbia extract	0.5
Propylene glycol	4.5
Emulsifying wax	9.0
Beeswax	4.0
Span 80	2.0

Procedure:

1) Emulsifying wax and beeswax are melted together and mixed.
2) Span 80 is added to the material of step 1 and mixed.
3) Euphorbia extract is dissolved in propylene glycol at 40-45^{0}C with constant stirring.
4) The material of step 3 is added to the material of step 2 and mixed well.
5) The material of step 4 is poured into suppository moulds and cooled.
6) Suppositories thus formed are removed from moulds and packed suitably.

EXAMPLE 21

Ingredient	**gm/10 units**
Purified euphorbia extract	0.5
Propylene glycol	1.5
Witepsol – 45	16.0
Cetyl alcohol	1.0
Beeswax	1.0

Procedure:

1) Cetyl alcohol, beeswax and Witepsol-45 are melted together.
2) Euphorbia extract is dissolved in propylene glycol at 40-45^{0}C with constant stirring.
3) The material of step 2 is added to the material of step 1 and mixed well.
4) The material of step 3 is poured into suppository moulds and cooled.
5) Suppositories thus formed are removed from moulds and packed suitably.

EVALUATION OF CLINICAL EFFICACY OF CAPSULES AND CREAM

The effective dose of extract of *Euphorbia prostrata* in nociceptive and inflammatory animal models varied from 5–20 mg/kg in mice and rats. Moreover, the maximum tolerable dose is more than 2000 mg/kg in mice and rats. Based on body surface area to body weight ratios, the expected dose of extract of *Euphorbia prostrata* for human studies could be in between 50 – 200 mg for 60 kg human being (Paget and Barnes, 1964; Freireich *et al.*, 1966). It is observed that the maximum human therapeutic dose (200 mg for 60 kg human being) is approximately 57 and 112 times less than the maximum dose employed in the acute toxicity studies in mice and rats respectively calculated based on body surface area to body weight ratios.

Results of the clinical trials conducted at RML and LNJP hospitals, Delhi (India)

The optimal dose, efficacy, safety and patient tolerability of 50 and 100 mg capsule formulations of extract of *Euphorbia prostrata* in hemorrhoidal attacks was evaluated in a double blind, placebo controlled, prospective, comparative and a randomized study. The duration of study was 8 months and protocol therapy was for 10 days.

A total of 125 patients entered the study, out of which 72 patients suffered from degree I hemorrhoids and 53 patients suffered from degree II hemorrhoids. The patients in each category were randomized into 3 treatment groups i.e. TDA, TDB and TDC (i.e. 50 mg, 100 mg and placebo capsules). All patients were evaluated on day 5 and day 10 of starting therapy. A follow up of 3 months was done.

The clinical examination was carried out to score the signs and symptoms i.e. proctorrhagia, anal discomfort, pain, anal discharge and proctitis at Day 0, Day 5 and Day 10. The degree of improvement on individual clinical parameters was also assessed from day 0, Day 5 and Day 10. The number of episodes of bleeding with bowel action and utilization of analgesics and topical medication as rescue medication was also assessed at Day 0, Day 5, and Day 10.

For statistical description, all patients were included in "Intent to treat" analysis. Kruskal Wallis test and Wilcoxan signed rank test for qualitative variables and paired t-test and one-way ANOVA for quantitative analysis were applied.

The study demonstrated that 100 mg capsule of extract of *Euphorbia prostrata* (TDB) and 50 mg capsule of extract of *Euphorbia prostrata* (TDA) was generally more effective than placebo (TDC) group in all the efficacy parameters of hemorrhoids.

All the groups tolerated the drugs well and showed minimal side effects. All the laboratory parameters were normal at baseline as well as at the end of the therapy in all the 3 treatment groups.

Previous studies have demonstrated the efficacy and safety of extract of *Euphorbia prostrata* in the treatment of hemorrhoids in an uncontrolled fashion. In this study, extract of *Euphorbia prostrata* in two doses i.e. 50 and 100 mg showed good efficacy in treatment of hemorrhoids. However clinically, TDB group (100 mg capsule) showed better results in overall therapeutic evaluation.

In Degree I hemorrhoids. certain parameters in TDA group showed better results. From the analysis it was found that out of the three treatment groups, maximum number of patients in TDB group underwent 5-day therapy. The prolonged 10-day therapy in TDA group might attribute to the better response in some parameters. However, at day 5, in few evaluation parameters i.e., and discomfort and proctorrhagia, complete recovery was found in maximum number of patients in TDB group.

When the assessment on the degree of improvement in overall signs and symptoms of hemorrhoidal attacks was done, highest number of patients in TDB group showed substantial improvement at both assessment day i.e. Day 5 and Day 10. Clinically TDB group showed the better decrease in bleeding episodes at both Day 5 and Day 10. At 3 months follow up; both treatment groups i.e. TDA and TDB groups were found to be quite effective in terms of non-reoccurrence of bleeding.

It was found that although bleeding reoccurred in slightly more number of patients in TDB group, no treatment was needed in highest number of patients in TDB than TDA group. It may be inferred from this that the intensity of bleeding was not so severe to require any treatment interference.

In Degree II Hemorrhoids, of all the treatment groups, TDB showed better results in anal discomfort and proctitis at Day 5. In other parameters i.e., proctorrhagia, anal discharge and pain at prolapse, both TDA and TDB were comparable but better than TDC clinically.

Also, at Day 5, TDB proved to be a better drug in bringing about complete disappearance of signs and symptoms of hemorrhoidal attacks. The better results shown by TDB group at Day 5 than at day 10 may be attributed to the fact that of all the treatment groups, highest number of patients in TDB groups underwent 5-day therapy and did not require prolonged therapy of 10 days. At 3 months follow up analysis; TDB was found to be better in all the aspects.

The use of rescue medication in Degree I and Degree II hemorrhoids was seen in lesser number of patients in TDB group at the end of therapy i.e. Day 10. The impact of rescue medication might also explain some better results seen with lesser dose of 50 mg (TDA) as compared to high dose 100 mg (TDB) in few parameters.

We claim:

1. A pharmaceutical composition for the treatment of anorectal or colonic disease such as hemorrhoids, fissures, cracks, fistulas, abscesses, inflammatory bowel disease, and the like comprising of an extract of the plant *Euphorbia prostrata* containing flavonoids and phenolic compounds, wherein the flavonoids are apigenin-7-glycoside, 1 - 4% by weight; luteolin-7-glycoside, 0.3 - 2% by weight; and apigenin, luteolin and quercetin, 0.001 – 0.3% by weight; and wherein the phenolic compounds are ellagic acid, 1-15% by weight; gallic acid, 1 – 12% by weight and tannins, 1 - 10% by weight, with pharmaceutically acceptable carrier(s)/base(s); optionally with additional therapeutic agent(s) prepared by a process comprising of the following steps.

   a. drying the plant *Euphorbia prostrata* under controlled conditions of temperature and humidity,

   b. making a powder from the dried plant,

   c. extracting the dry course powder with a polar solvent repetitively to form an extract,

   d. distilling the extract,

   e. washing the concentrated extract with a non-polar organic solvent, and

   f. drying the washed extract to produce the desired pharmaceutically acceptable extract capable of being used along with suitable carrier(s)/base(s).

2. The pharmaceutical composition according to claim 1 wherein the process for the manufacture of the extract further comprises:

   a. re-extracting the washed polar extract in a medium polarity organic solvent,

   b. distilling the extract,

   c. dehydrating the extract, and

   d. drying the extract to produce the desired pharmaceutically acceptable extract capable of being used along with a suitable carrier(s)/base(s).

3. The pharmaceutical composition as claimed in claim 1 and 2, wherein the extract comprises preferably 2.5 – 3.5% by weight apigenin-7-glycoside, 0.5 – 1.5% by weight

luteolin-7-glycoside, 0. 05 – 0.2% by weight apigenin, luteolin and quercetin, 4 - 15% by weight ellagic acid, 4 – 12% by weight gallic acid and 3 - 8% by weight tannins.

4. The pharmaceutical composition as claimed in claim 1, wherein the composition further comprises pharmaceutically acceptable carrier(s)/base(s).

5. The pharmaceutical composition as claimed in claim 3, wherein the composition further comprises pharmaceutically acceptable carrier(s)/base(s).

6. The pharmaceutical composition as claimed in claim 1, comprising additional therapeutic agent(s), selected from astringents, anesthetics, vasoconstrictors, protectants, counterirritants, keratolytics, anti-cholinergics, wound healing agents and anti-microbial agents, or their pharmaceutically acceptable salts; used either alone or in combination thereof.

7. The pharmaceutical composition as claimed in claim 3, comprising additional therapeutic agent(s), selected from astringents, anesthetics, vasoconstrictors, protectants, counterirritants, keratolytics, anti-cholinergics, wound healing agents and antimicrobial agents, or their pharmaceutically acceptable salts; used either alone or in combination thereof.

8. The pharmaceutical composition as claimed in claim 6, wherein the additional therapeutic agent is an astringent.

9. The pharmaceutical composition as claimed in claim 8, wherein the astringent is selected from calamine, zinc oxide, hamamelis water, bismuthresorcinol compound, bismuth subgallate, Peruvian balsam, aluminium chlorohydroxy allantoinate, tannic acid, and the like; used either alone or in combination thereof.

10. The pharmaceutical composition as claimed in claim 8, wherein the amount of the astringent varies between 0.2% and 60% by weight.

11. The pharmaceutical composition as claimed in claim 6, wherein the additional therapeutic agent is an anesthetic.

12. The pharmaceutical composition as claimed in claim 11, wherein the anesthetic is selected from benzocaine, diperomon, pramoxine, camphor, dibucaine, phenol, tetracaine, phenacaine, and the like; used either alone or in combination thereof.

13. The pharmaceutical composition as claimed in claim 11, wherein the amount of the anesthetic varies between 0.25% and 25% by weight.

14. The pharmaceutical composition as claimed in claim 6, wherein the additional therapeutic agent is a vasoconstrictor.

15. The pharmaceutical composition as claimed in claim 14, wherein the vasoconstrictor is selected from ephedrine or phenylephrine; used either alone or in combination thereof.

16. The pharmaceutical composition as claimed in claim 14, wherein the amount of the vasoconstrictor varies between 0.005% and 1.5% by weight.

17. The pharmaceutical composition as claimed in claim 6, wherein the therapeutic agent is a counterirritant.

18. The pharmaceutical composition as claimed in claim 17, wherein the counterirritant is menthol and is present an amount between 0.25 and 2.5%.

19. The pharmaceutical composition as claimed in claim 6, wherein the therapeutic agents is a protectant.

20. The pharmaceutical composition as claimed in claim 19, wherein the protectant is selected from aluminium hydroxide gel, calamine, cocoa butter, cod or shark liver oil, starch, white petroleum, wool alcohol, zinc oxide, vegetable or castor oil, polyethylene glycol, propylene glycol, and the like; used either alone or in combination thereof.

21. The pharmaceutical composition as claimed in claim 19, wherein the protectant is present in an amount between 5.0% and 88.0% by weight.

22. The pharmaceutical composition as claimed in claim 6, wherein the therapeutic agent is a wound healing agent.

23. The pharmaceutical composition as claimed in claim 22, wherein the wound healing agent is selected from vitamin A, vitamin D, Peruvian balsam, cod liver oil and the like; used either alone or in combination thereof.

24. The pharmaceutical composition as claimed in claim 22, wherein the vitamin A and vitamin D are present in an amount between 0.005% to 0.04% by weight, the Peruvian

balsam is present in an amount between 0.5% to 2.5% by weight and cod liver oil is present in an amount between1.0% to 6.0% by weight.

25. The pharmaceutical composition as claimed in claim 6, wherein the therapeutic agent is an antimicrobial agent.

26. The pharmaceutical composition as claimed in claim 25, wherein the antimicrobial agent is selected from benzethonium chloride, benzalkonium chloride, boric acid, 8-quinolinol benzoate, secondary amyltricresols, cetylpyridinium chloride, phenol, menthol, chlorothymol, camphor and 8-hydroxyquinoline sulfate and the like; used either alone or in combination thereof.

27. The pharmaceutical composition as claimed in claim 25, wherein the antimicrobial agent is present in an amount between 0.02% and 40% by weight.

28. The pharmaceutical composition as claimed in claim 6, wherein the therapeutic agent is a keratolytic.

29. The pharmaceutical composition as claimed in claim 28, wherein the keratolytic is selected from aluminium chlorohydroxy allantoinate and resorcinol, used either alone or in combination thereof.

30. The pharmaceutical composition as claimed in claim 28, wherein the keratolytic is present in an amount between 0.2% and 3.5% by weight.

31. The pharmaceutical composition as claimed in claim 6, wherein the therapeutic agents are anticholinergics.

32. The pharmaceutical composition as claimed in claim 31, wherein the anticholinergics are selected from atropine or other solanaceous type alkaloids; used either alone or in combination thereof.

33. The pharmaceutical composition as claimed in claim 31, wherein the amount of the anti-cholinergic varies between 0.02% and 0.1% by weight.

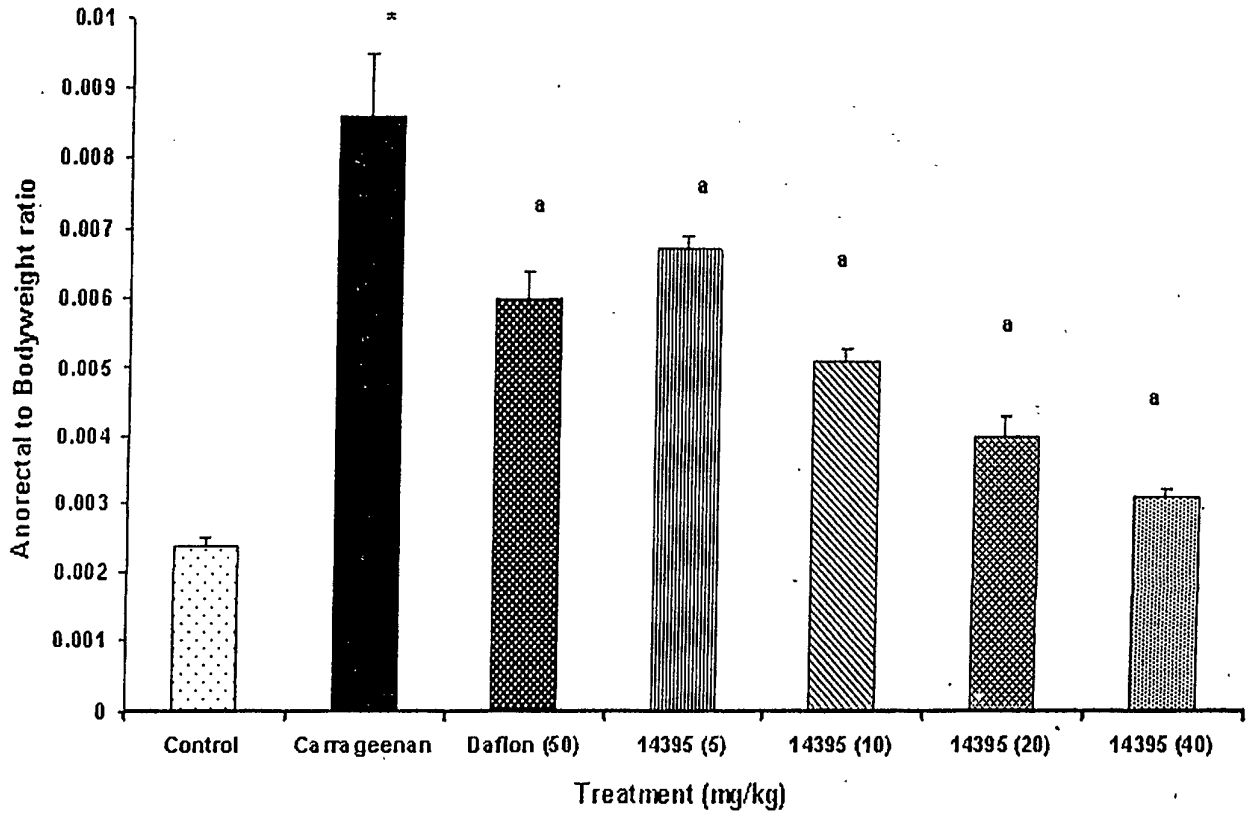
34. The pharmaceutical composition as claimed in claim 1, wherein the composition is in the form of a cream, ointment, solution, spray, foam, suppository, medicated pad, bandage, powder, suspension, film, flake, oral hard gelatin capsules, soft gelatin capsules, tablets

(coated and uncoated), modified release dosage form, liquid, lozenges, buccal or sublingual dosage form, wafers, caplets, or parenteral dosage form to be infiltered at the site of the injection.

Abstract

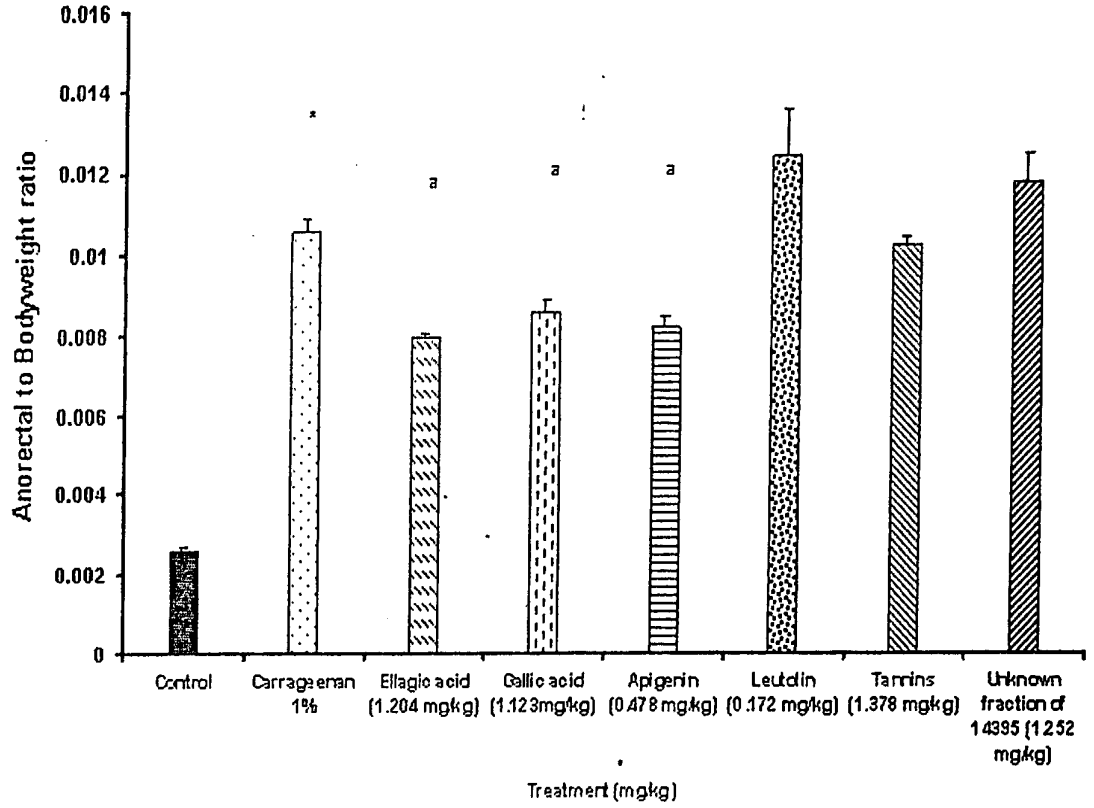
Novel compositions and a method for treating anorectal diseases including hemorrhoids and colonic diseases with long term effectiveness and a low prolapse rate are disclosed. The compositions can be orally administered as well as uniformly applied to the affected region. The composition comprise of flavonoidal and phenolic constituents extracted from the plant *Euphorbia prostrata* that possess anti-inflammatory, analgesic, haemostatic and wound-healing properties.

Figure 1 : Effect of *Euphorbia prostrata* extract (14395) against carrageenan-induced hemorrhoids in rats



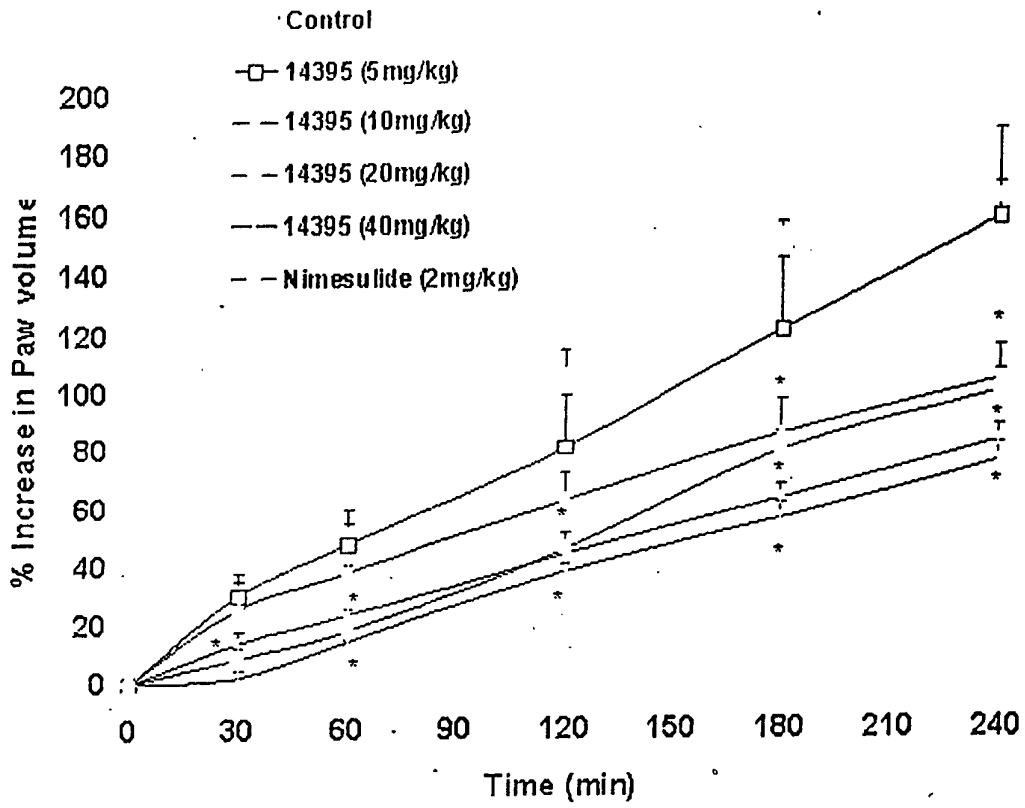
*$p < 0.05$ as compared with control group, [a] $p < 0.05$ as compared with carrageenan -treated group,

Figure 2 : Effect of individual component of *Euphorbia prostrata* extract (14395) against carrageenan-induced hemorrhoids in rats



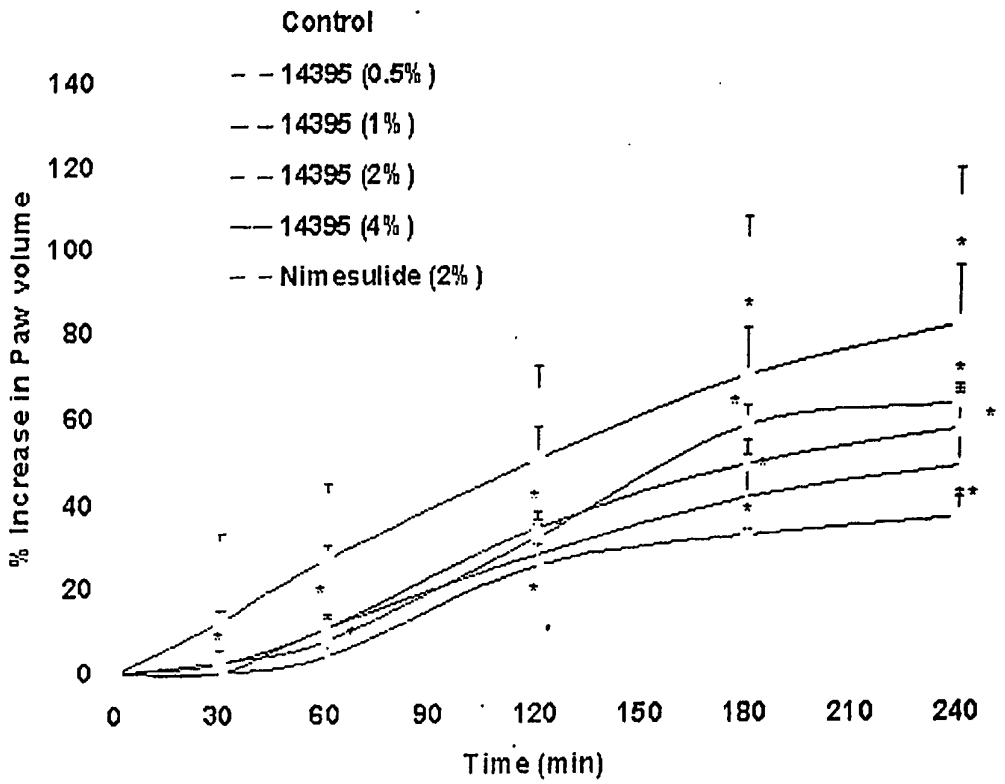
*$p < 0.05$ as compared with control group, [a] $p < 0.05$ as compared with carrageenan -treated group

Figure 3 : Effect of *Euphorbia prostrata* extract (14395) against carrageenan-induced paw oedema (oral)



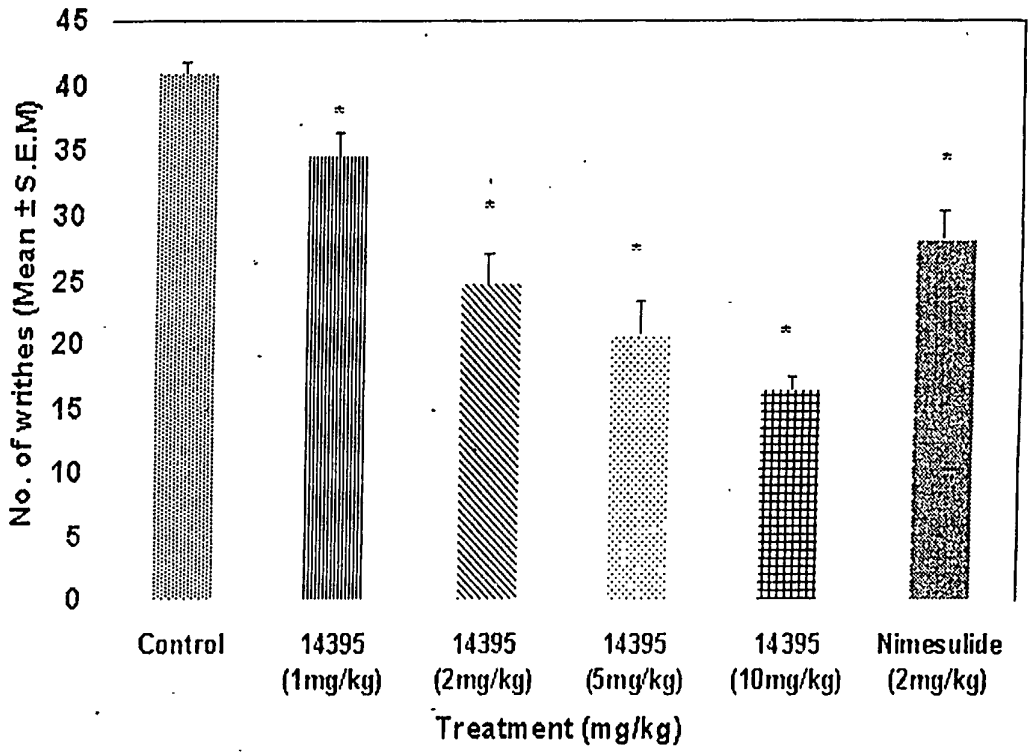
$^{*}p < 0.05$ as compared with control group

Figure 4 : Effect of *Euphorbia prostrata* extract (14395) against carrageenan-induced paw oedema (topical).



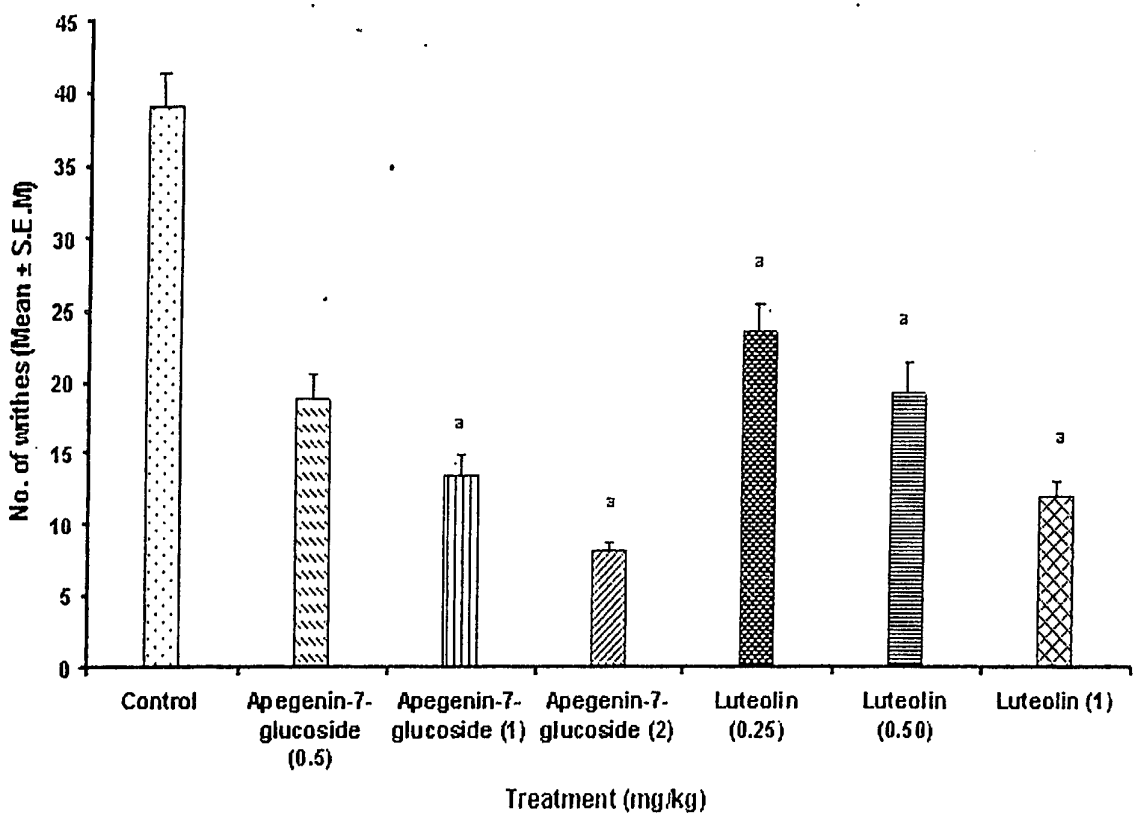
$p < 0.05$ as compared with control group

Figure 5 : Effect of *Euphorbia prostrata* extract (14395) against acetic acid-induced chemonociception in mice.



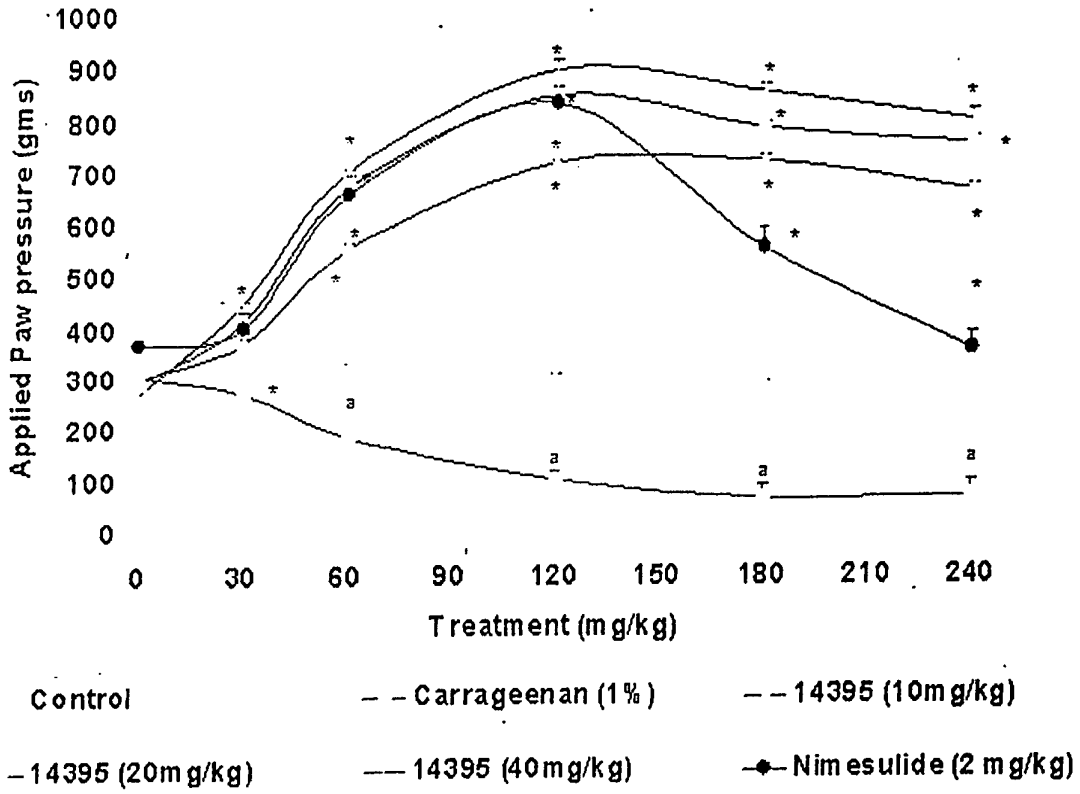
** p < 0.05 as compared to the control group*

Figure 6 : Effect of principal components of *Euphorbia prostrata* extract (14395) against acetic acid-induced chemonociception in mice.



** p < 0.05 as compared to the control group*

Figure 7 : Effect of *Euphorbia prostrata* extract (14395) against carageenan-induced hyperalgesia in rats.



[a] *p < 0.05 as compared to the control group*

[*] *p < 0.05 as compared to the carrageenan treated group*

Figure 8 : Effect of *Euphorbia prostrata* extract (14395) on bleeding time in liver incision model.

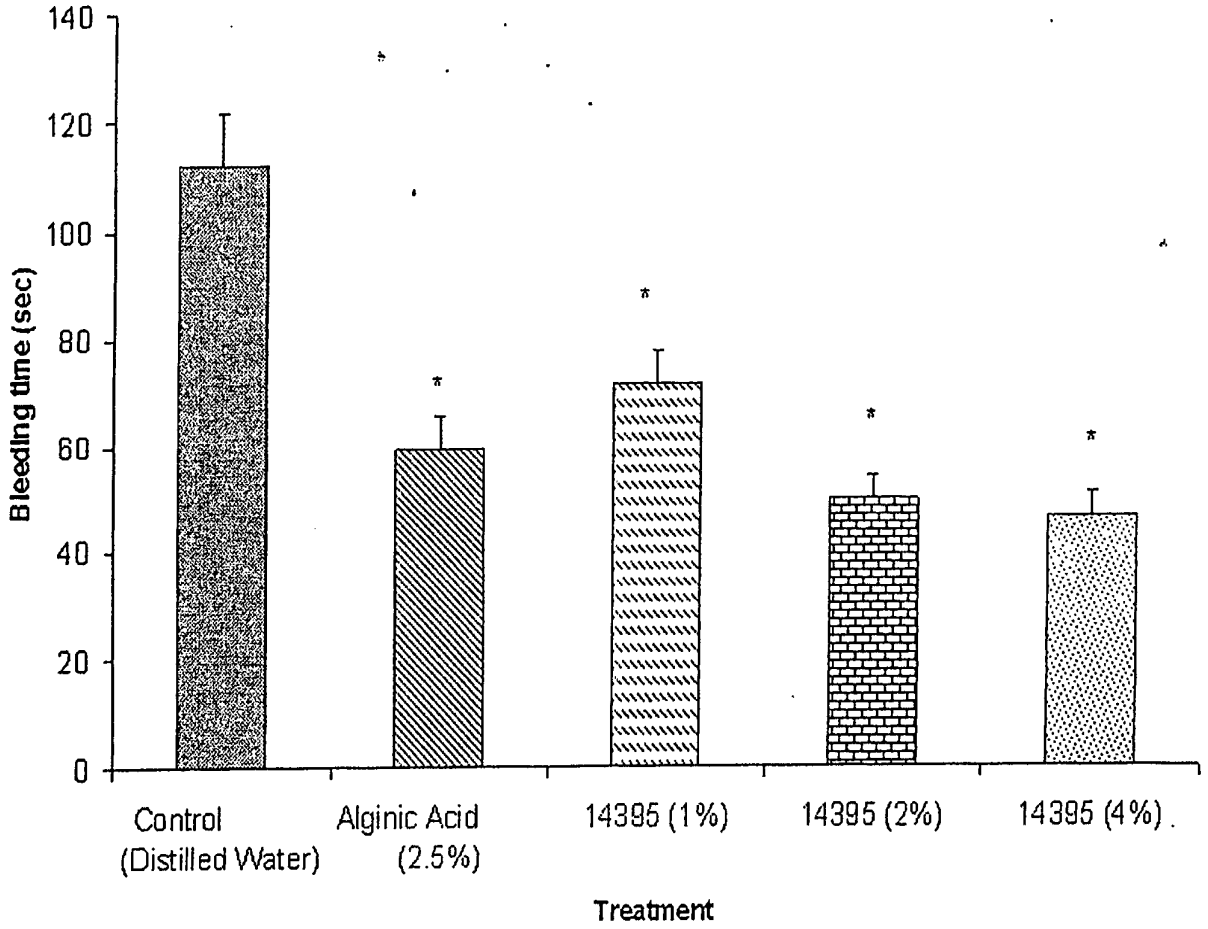
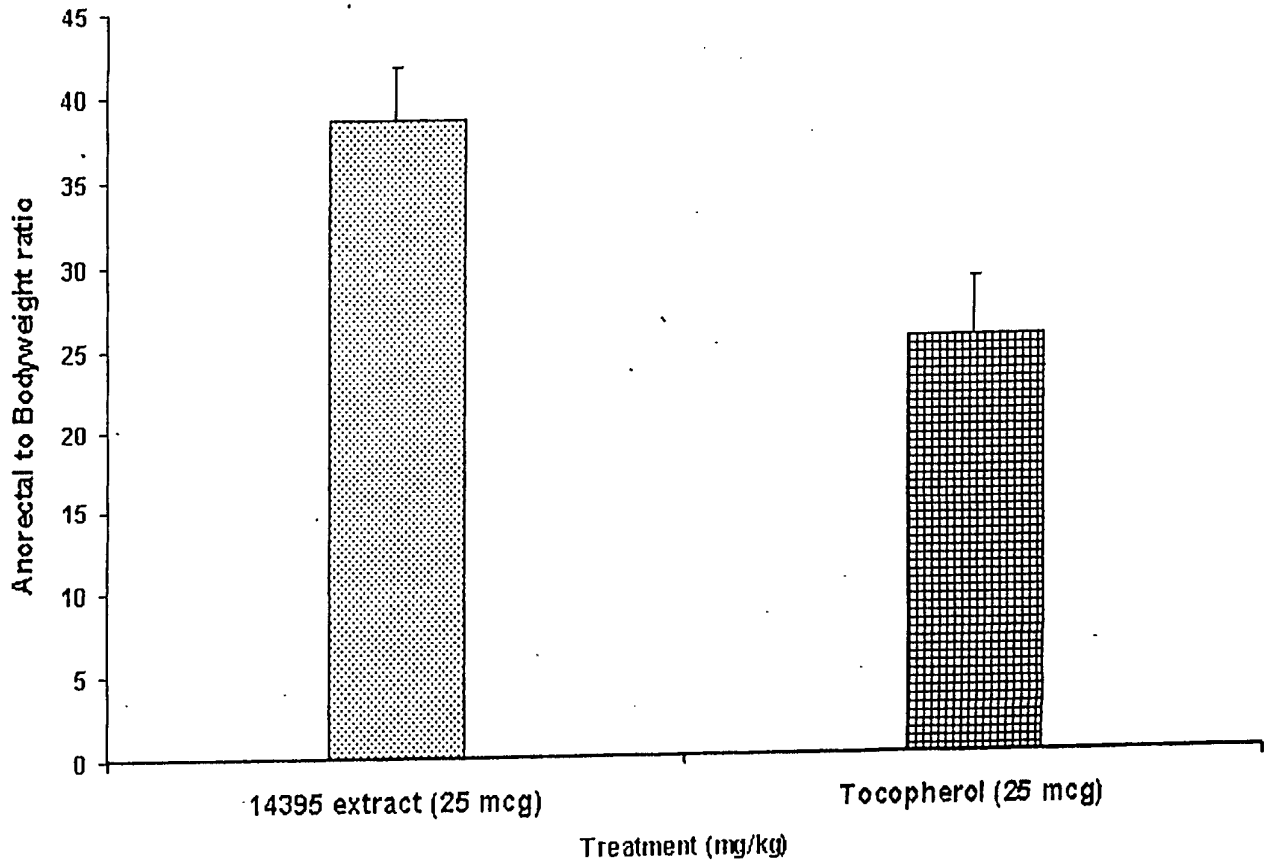


Figure 9 : *In vitro* superoxide radical scavenging activity of *Euphorbia prostrata* extract (14395).



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